PRACTICAL MANUAL

on

Principles of Vegetable Breeding

Course No. HVS-504; Credit Hrs. 3(2+1)
For

M.Sc. (Horticulture) Vegetable Science)

Ist-year (1st Semester)

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CONTENT

Experiment No. 1

Objective: Vegetable breeder's kit for plant breeding

Materials required: Forceps, scissors, alcohol, tags, pencil and butter paper bags

Equipments Uses:

1) **Scissor**: It is required for removal of unwanted leaves, buds, etc.

2) **Needle Long and pointed needle**: is required to open the small flower buds.

3) **Forceps/Tweezers**: Fine forceps with long point is required for removing the anthers during emasculation and to transfer the pollen grains from male flowers to the stigma of emasculated female flowers

4) **Brush Hair brush**: is required for collecting the pollen grains and dusting on the stigma of emasculated female flowers.

5) **Spirit bottle**: A small bottle of alcohol or spritis needed to sterilize the scissors, needles, forceps and brush as well as hands during the crossing work

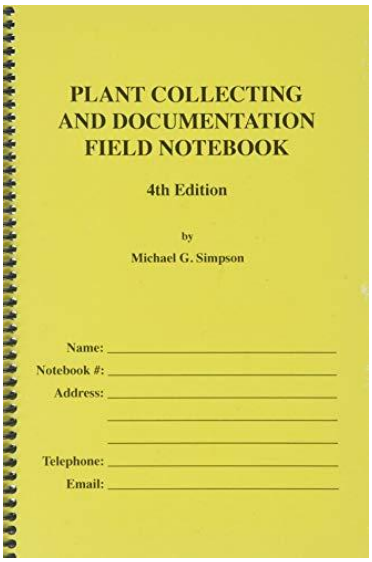
6) **Magnifying Lens** It is useful in emasculating small flower buds and it is also required to observe the stigma of emasculated flower to confirm 2 that stigma does not carry any anthers or pollen grains

7) **Bags** Bags are required to cover the flowers or inflorescences prior to pollination and after the pollination. Bags are of different kinds and sizes are used depending upon types of crops and size of flowers, viz., brown paper bag, muslin cloth bag, parchment paper bag, etc.

8) **Labels** Paper or aluminum labels are used to write the necessary information. The information on the label should be written with non-copying pencil by hands

9**) U - Pins or Wire rings** They are used for holding the paper bag kept on the flowers or inflorescences.

10) **Field Notebook** All the important observations required to be takenduring the breeding works are written in the field notebook. This notebook should have the information/record of number of crosses, date of selfing or emasculation, flowering, maturity, number of seeds set in crossed flower and about the plant selected in segregating generations



Exercise:

Practical exercise on use of breeding tools in vegetable crops

Experiment No. 2

Objective: Techniques of selfing and crossing in vegetable crops

Introduction: Selfing and crossing are the essential procedures in crop improvement process. The exact procedures used to ensure self or cross-pollination of specific plants will depend on the floral structure and normal manner of pollination. Generally effecting cross-pollination in a strictly self-pollinating species is more difficult than vice-versa because for instance preventing self-pollination occurring inside the unopened flowers is cumbersome.

Selfing

In the selfing of cross-pollinated species, it is essential that the flower are bagged or otherwise protected to prevent natural cross-pollination. The exact procedure that breeder may use to ensure self or cross pollination of specific plants will depend on the particular species with which he is working. The structure of the flowers in the species determine manner of pollination. For these reasons, the breeder should acquaint himself with the flowering habit of the crop.

In tomato, Hand emasculation and hand pollination is to be practiced. Two parents having good combining ability are selected. Varieties maintained by selfing can be taken as parents. In a few cases 2-3 years selfing is done to maintain the purity. A large number of such parents crossed in different combination and resultant hybrids evaluated at one or more location. In a few cases sca is studied to select a potential hybrid. Practically, superiority is judged by per se performance. Hybrid seeds largely produced by hand emasculation and hand pollination. Seed plants and pollen parents are grown under healthy condition in 12:1 ratio. Emasculation of seed parent is taken 12-15 hr before anthesis, by forceps, needle or fingernail. Generally emasculation is done in the afternoon and pollination is done in the following morning. Pollen collected from anthers by needle, forceps or electric vibrator. Generally fresh pollen is used. Pollen can be stored for 2-3 days under normal condition. Soon after pollination, 2-3 sepals of the pollinated flowers are removed for easy identification. (No need to put bag or cotton). 4-5 days - ovary start swelling. More than 90% fruit set after hybridization.

Emasculation

Removal of stamens or anthers or killing the pollen of a flower without the female reproductive organ is known as emasculation. In bisexual flowers, emasculation is essential to prevent of self-pollination. In monoecious plants, male flowers are removed; (cucurbits) or male inflorescence is removed (bottle gourd). In species with large flowers e.g. (okra, tomato, brinjal, chilli, pumpkin) hand emasculation is accurate and it is adequate.

Methods of Emasculation

1. Hand Emasculation

In species with large flowers, removal of anthers is possible with the help of forceps. It is done before anther dehiscence. It is generally done between 4 and 6 PM one day before anthers dehisce. It is always desirable to remove other young flowers located close to the emasculated flower to avoid confusion. The corolla of the selected flower is opened with the help of forceps and the anthers are carefully removed with the help of forceps. Sometimes corolla may be totally removed along with epipetalous stamens. e.g. tomato, brinjal, chilli.

2. Suction Method

It is useful in species with small flowers. Emasculation is done in the morning immediately after the flowers open. A thin rubber or a glass tube attached to a suction hose is used to suck the anthers from the flowers. The amount of suction used is very important which should be

sufficient to suck the pollen and anthers but not gynoecium. In this method considerable self-pollination, upto 10% is like to occur. Washing the stigma with a jet of water may help in reducing self-pollination, However self pollination can not be eliminated in this method.

3. Hot Water Treatment

Pollen grains are more sensitive than female reproductive organs to both genetic and environmental factors. In case of hot water emasculation, the temperature of water and duration of treatment vary from crop to crop. It is determined for every species. For sorghum 42-480C for 10 minutes is found to be suitable. In the case of rice, 10 minutes treatments with 40-440C is adequate. Treatment is given before the anthers dehiscence and prior to the opening of the flower. Hot water is generally carried in thermos flask and whole inflorescence is immersed in hot water.

4. Cold Treatment

Cold treatment like hot water treatment kills the pollen grains without damaging gynoecium.

6. Genetic Emasculation

Genetic/ cytoplasmic male sterility may be used to eliminate the process of emasculation. This is useful in the commercial production of hybrids in cole crops, chilli, onion, muskmelon etc.

In many species of self-incompatible cases, also emasculation is not necessary, because self-fertilization will not take place. Protogyny will also facilitate crossing without emasculation.

7. Use of Gametocide

Also known as chemical hybridizing agents (CHA) chemicals which selectively kills the male gamete without affecting the female gamete. Eg. FW450 in tomato.

Bagging

Immediately after emasculation the flower or inflorescence enclosed with suitable bags of appropriate size to prevent random cross-pollination.

Pollination

The pollen grains collected from a desired male parent should be transferred to the emasculated flower. This is normally done in the morning hours during anthesis. The flowers are bagged immediately after artificial crossing.

Tagging

The flowers are tagged just after bagging. They are attached to the inflorescence or to the flower with the help of a thread. The following may be recorded on the tag with pencil.

1. Date of emasculation
2. Date of pollination
3. Parentage
4. No. of flowers emasculate

Exercise:

Selfing and crossing techniques in onion

Selfing and crossing techniques in cabbage

Experiment No. 3

Objective: To study about floral biology and hybrid breeding of tomato

Botanical Name: ……..

Chromosome No.: ……..

Family: ……

Inflorescence: Axillary or terminal condensed cymes.

Flower: Pedicellate, bracteate, hermaphrodite, complete, actinomorphic, pentamerous, hypogynous.

Calyx: Sepals 5, gamosepalous, campanulate, sepals free above and fused below, green, hairy, imbricate aestivation, inferior.

Corolla: Petals 5, gamopetalous, campanulate, tube hairy, twisted aestivation, inferior.

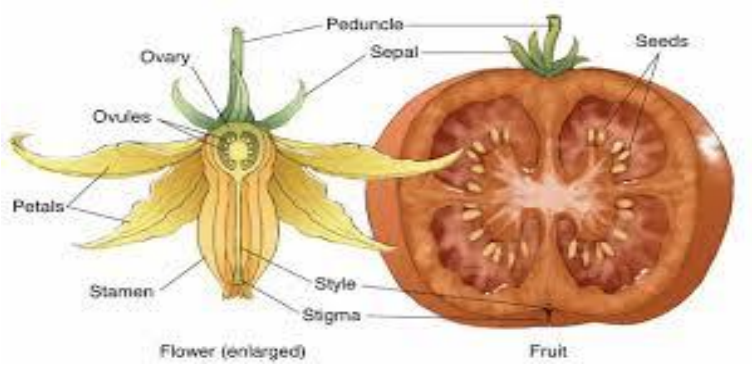
Androecium: Stamens 5, polyandrous, epipetalous, alternating with the petals, filaments long, anthers basifixed, dithecous, introrse.

Gynoecium: Bicarpellary, syncarpous, ovary superior, obliquely placed, bilocular; swollen axile placentation; ovules many; style long, slightly, twisted, stigma capitate, bilobed.

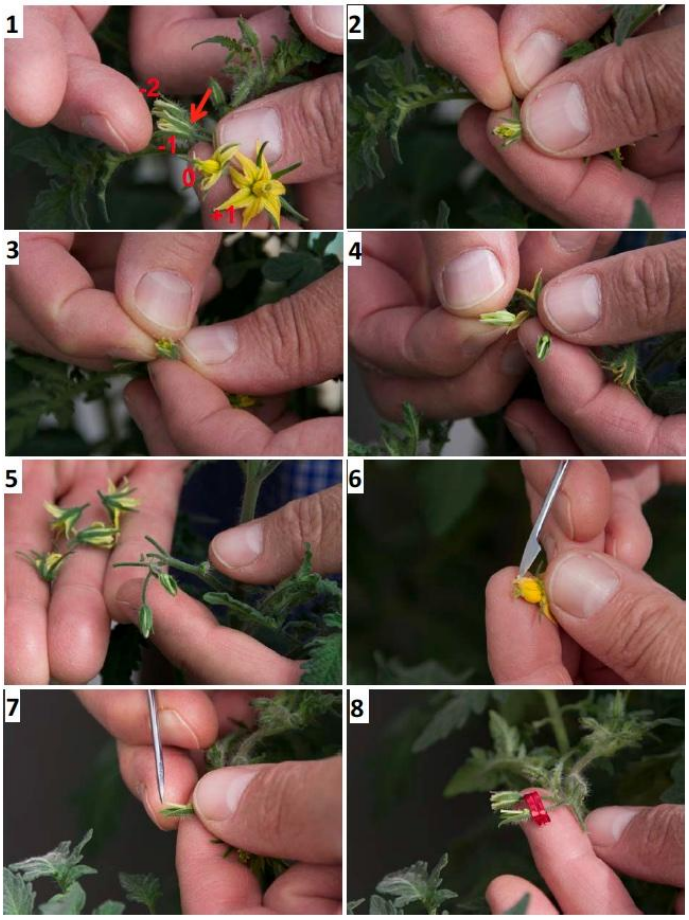
Floral formula:

$$\mathbf{Br} \oplus \varnothing \ \mathbf{K}_{(5)} \overset{\frown}{\mathbf{C}_{(5)}} \mathbf{A}_{5} \ \underline{\mathbf{G}_{(2)}}.$$

Draw the floral diagram of tomato



Emasculation and pollination for hybrid breeding in tomato



Write the protocol of emasculation and pollination:

Experiment No. 4

Objective: To study about genic male sterility in hybrid breeding of chilli

Botanical Name: ……. .

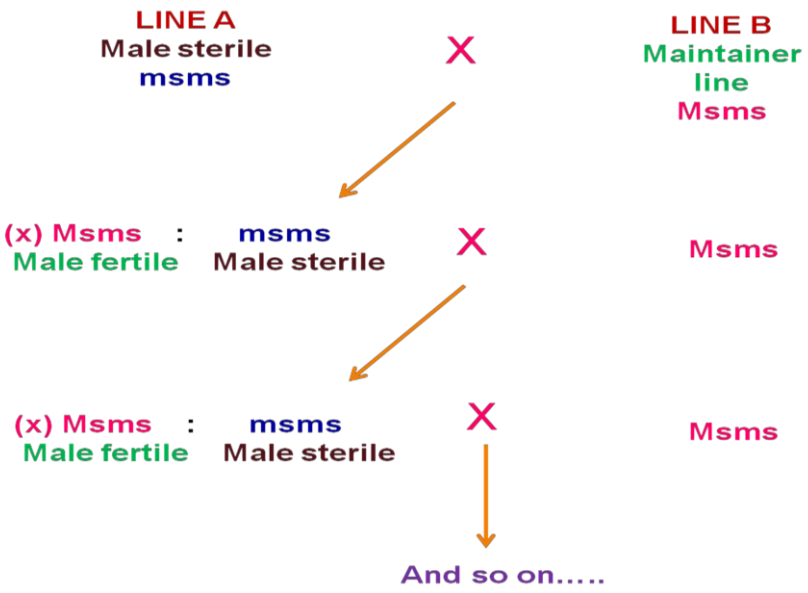
Chromosome No.: ……

Family: ……..

Male sterility in chilli: Commercial hybrid seed in chilli are produced either by hand-emasculation or by exploiting the male sterility. Both the nuclear or genic male sterility (GMS) and the cytoplasmic male sterility (CMS) have been reported and utilized for hybrid development. Limitation of the GMS is that the progeny segregates into male sterile and male fertile plants, and the 50% male fertile plants have to be identified and removed from the seed production block. This is tedious and time consuming, and the seed is prone to genetic impurities resulting from improper identification and self-pollination.

The CMS in Capsicum was first reported by Peterson (1958) in 'PI 164835', an introduction from India. Various S-type cytoplasms reported in Capsicum spp. might be identical. In CMS, the sterility is determined by interaction between the S-cytoplasm and the recessive nuclear restorer-of-fertility (rf) gene. The dominant (Rf) gene restores the fertility by suppressing the CMS-associated genes.

Maintenance of GMS lines in chilli:



Hybrid seed production in chilli using GMS system:

GMS based hybrids of chilli:

Experiment No. 5

Objective: To study about floral biology and hybrid breeding of eggplant

Botanical Name: ……. .

Chromosome No.: ……

Family: ……..

Brinjal belongs to the family Solanaceae and is known under the botanical name *Solanum melongena* L. The family contains 75 genera and over 2000 species, out of which, about 150-200 are tuber bearing and belong to section Tuberarium. The majority of species (about 1800) are non tuber bearing. Cytologica studies have indicated that basic chromosomal number 2n = 24 is same in almost all the varieties and species.

The common brinjal, to which large, round or eggshaped fruited forms belong, are grouped under var. *esculentum*. The long, slender types are included under var. *serpentinum* and the dwarf brinjal plants are put under var. *depressum.*

Floral biology and floral diagram:

Emasculation and pollination procedure in eggplant

Flower types in eggplant:

Long styled:

Medium styled

Pseudo short styled

True short styled

Experiment No. 6

Objective: To study about floral biology and CMS system in breeding of cauliflower

Botanical Name: ……. .

Chromosome No.: ……

Family: ……..

Floral biology: All the cole crops have evolved from common ancestor wild cabbage (*B. oleracea* var. *sylvestris* L.) (Fig. 1). A cabbage flower has four sepals, four petals, six stamens in tetradynamous condition (two short and four long stamens) and a bicarpellary ovary which is superior and has a false septum. Ovules are attached on both the side of septum. Two active nectarines are located between the bases of short stamens and ovary. The buds open under pressure of rapidly growing petals and become fully expanded in about 12 hrs. Flowers are slightly protogynous and cabbage is naturally cross pollinated due to sporophytic self-incompatibility. Pollination is brought about by bees and flies. Bud pollination is effective to achieve selfing. For cross-pollination flower buds expected to open within 1-2 days are emasculated and are pollinated immediately with desired pollen using a brush/ flower stamens.

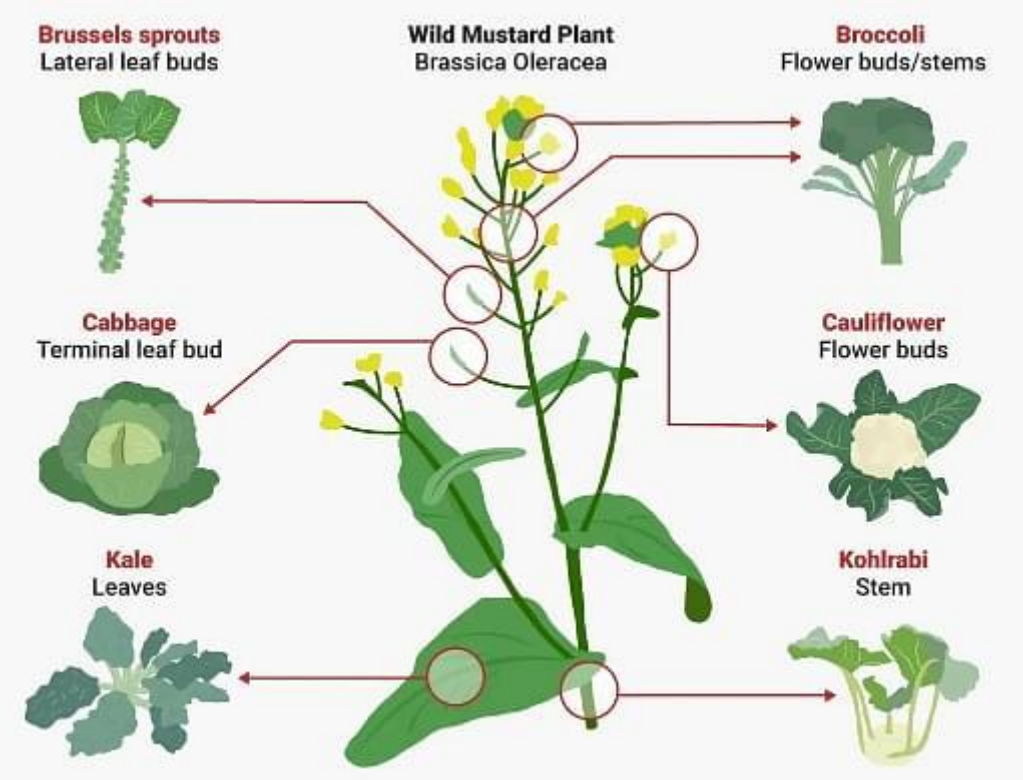
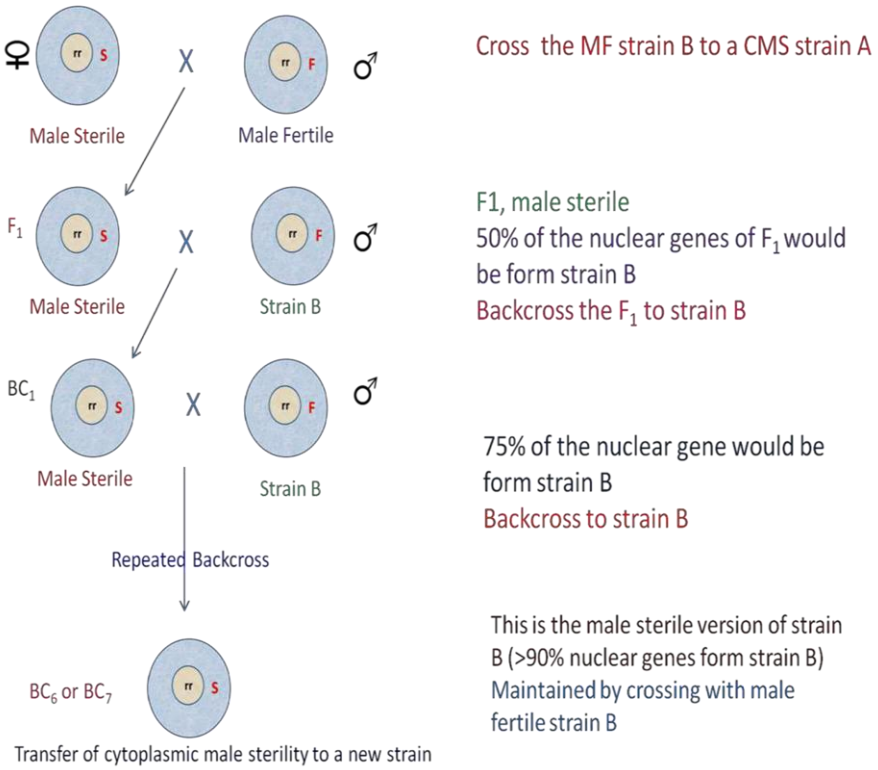


Figure 1: Evolution of cole crops

Bud pollination:

Cytoplasmic male sterility in cauliflower: Cytoplasmic male sterility (CMS) system is an economically important trait which has been commercially exploited for hybrid seed production in different vegetable crops such as snowball cauliflower. It represents maternal inheritance of inability of a plant to produce or shed viable pollen grains due to novel proteins produced by expression of unusual mitochondrial genes. There are different types of male sterile cytoplasms for Brassica crops including Ogu from wild Japanese radish.

Maintenance of CMS lines:



Protocol for hybrid breeding using CMS in Cauliflower:

Experiment No. 7

Objective: To study about floral biology and petaloid type male sterility in carrot

Botanical Name: ……. .

Chromosome No.: ……

Family: ……..

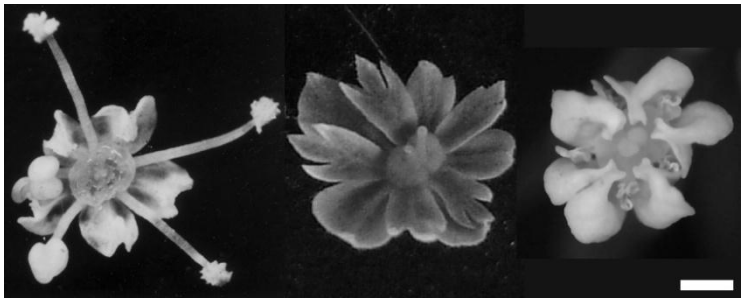
Floral biology:

The inflorescence of carrot is a compound umbel. A primary umbel can have over 1000 flowers at maturity, whereas secondary, tertiary and quaternary umbels bear fewer flowers. Floral development is centripetal i.e. the flowers to dehisce first are on the outer edges of the outer umbellets. Carrot is protandrous. After straightening of filament, the pollen is shed and stamens quickly fall. After this, the petals open fully and the style elongates. The style is divided into two parts. The petals of petaloid plants are persistent unlike those of brown-anther, male sterile plants. Flowers are epigynous. There are five small sepals, five petals, five stamens and two carpels. Emasculation is laborious and time consuming. As soon as the first bud in an umbel opens, the whole umbel of the female parent is bagged in a muslin/cloth bag. The flowers are removed daily until peak flowering has reached. Anthers are removed from the early opening outer flowers in the outer whorl of umbellets until sufficient flowers are emasculated.

Unopened central florets in the emasculated umbellets and all late flowering umbellets are removed. Thus, only the emasculated flowers are left on the female inflorescence inside the bag. A pollen bearing umbel from previously protected male plant is inserted into the bag of the female parent along with some house-flies to ensure pollination. Daily for a few days in the morning, the male umbel is gently rubbed against the emasculated umbel to enhance artificial cross-pollination.

Sometimes, 1-2 flowering umbels of both the parents are enclosed in the same cloth cage along with some house-flies. Seed from each parent is sown in adjacent rows. The hybrids and the parents could be identified (not always) and necessary roguing done to remove the selfed plants.

Types of Male sterility in carrot



Hybrid seed production procedure in carrot using pt-CMS system

Floral diagram of carrot

Experiment No. 8

Objective: To study about floral biology, selfing and crossing techniques in radish

Botanical Name: …… .

Chromosome No.: ……

Family: ……..

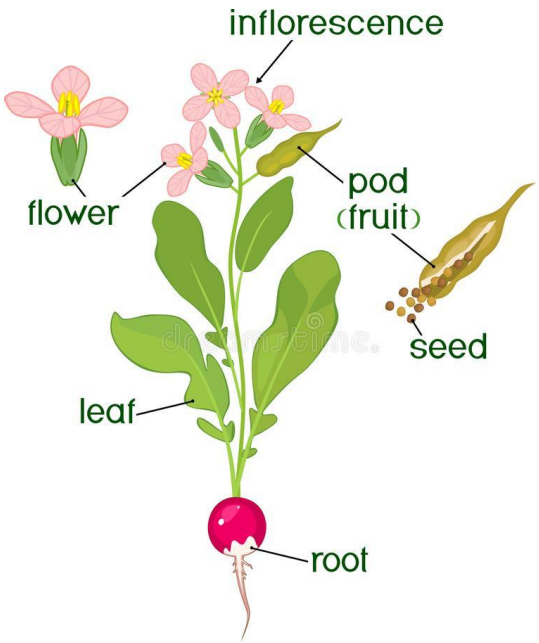
Floral biology:

The edible portion of radish develops from the primary root and hypocotyl the inflorescence is a typical terminal receme of cruciferae. The flowers are small, usually white in colour, sepals (four) are erect and petals (four) are clawed. Radish is cross pollinated due to sporophytic system of self- incompatibility. It shows considerable in breeding depression on selfing. It is entomophilous. It is pollinated mainly by wild honey bees and wild- flower flies. Stigma receptivity is maintained upto four days after anthesis.

Selfing: Selfing can be accomplished by bud-pollination. The flower buds are pollinated two days prior to opening by their own pollen be applying fresh pollen from previously bagged flowers of the same plant. Emasculation is not necessary in bud-pollination. After pollination, the buds are to be protected from foreign pollen by enclosing the particular branch bearing those buds in a muslin cloth bag.

Crossing: In crossing the same technique is used as in bud-pollination except that in the crossing, the buds of the female parent are emasculated a day prior to opening and are pollinated by pollen collected from the flowers of the male parent which were also bagged before opening. The artificial pollination is done by hand by shaking the pollen over the stigma directly from the freshly opened but previously bagged buds of male parent.

Draw the Floral diagram of radish:



Exercise: Write the hybrid breeding programme of radish using two self incompatible and cross compatible lines.

Experiment No. 9

Objective: To study about floral biology of beetroot

Botanical Name: ……. .

Chromosome No.: ……

Family: ……..

The earliest form of domesticated beet was leaf beet. The leaves were eaten and the roots were used for medical purposes only. According to Campbell (1979), this species is believed to have originated from *Beta maritima*, known as sea-beet which is indigenous to Southern Europe. The beetroot is closely related to sugar beet, with which it is cross-compatible. It has the almost unique characteristic (in the vegetable kingdom) of being wind-pollinated like its related species spinach, and is therefore a very prolific pollen producer. It belongs to family Chinopodiaceae. Garden beet is a biennial producing enlarged hypocotyl (roots) and a rossette of leaves in first year and flowers and seed in second year. Enlargement of hypocotyl is due to growth of several concentric vascular cambia which comprise the rings of beet.

Floral biology

It requires cold temperature (4-10°C) treatment for 2 weeks or longer for flower induction. The inflorescence is a large spike. The flowers are small, inconspicuous without corolla, but with green calyx which becomes thicker and covers the seed completely. This forms what is called the beet seed or multi-germ seed which, botanically is a fruit containing usually 2-6 seeds. The true seeds are small, kidney shaped and brown.

Selfing and crossing technique in beetroot:

Floral diagram

Experiment No. 10

Objective: To study about floral biology and crossing techniques in onion

Botanical Name: ……. .

Chromosome No.: ……

Family: ……..

Onion *Allium cepa L*. is an important vegetable crop grown throughout the world. It is a cool season vegetable. It is grown for its bulbs.

Floral biology:

- The flower structure is called an umbel which is an aggregate of many small inflorescences (cymes) of 5 to 10 flowers, each of which opens in a definite order causing flowering to be irregular and to last for two or more weeks.
- Each individual flower contains 6 stamens, 3 carpels united into one pistil and 6 perianth segments.
- The pistil contains 3 locules each of which has 2 ovules.
- The flower also contains nectarines which secrete nectar to attract insects for cross-pollination.
- The flowers are protandrous and anthers shed pollen over a period of 3-4 days prior to the time when full length of style is attained. Anthesis occurs in early morning (6-7 AM).
- Anther dehiscence is between 7.00AM and 5.00 PM and on next day also with peak between 9.30AM and 5.00 PM. Pollen fertility is maximum on the day of anthesis.
- Thus, stigma becomes receptive 3-4 days after shedding of pollen grains and protandry leading to favour cross pollination.

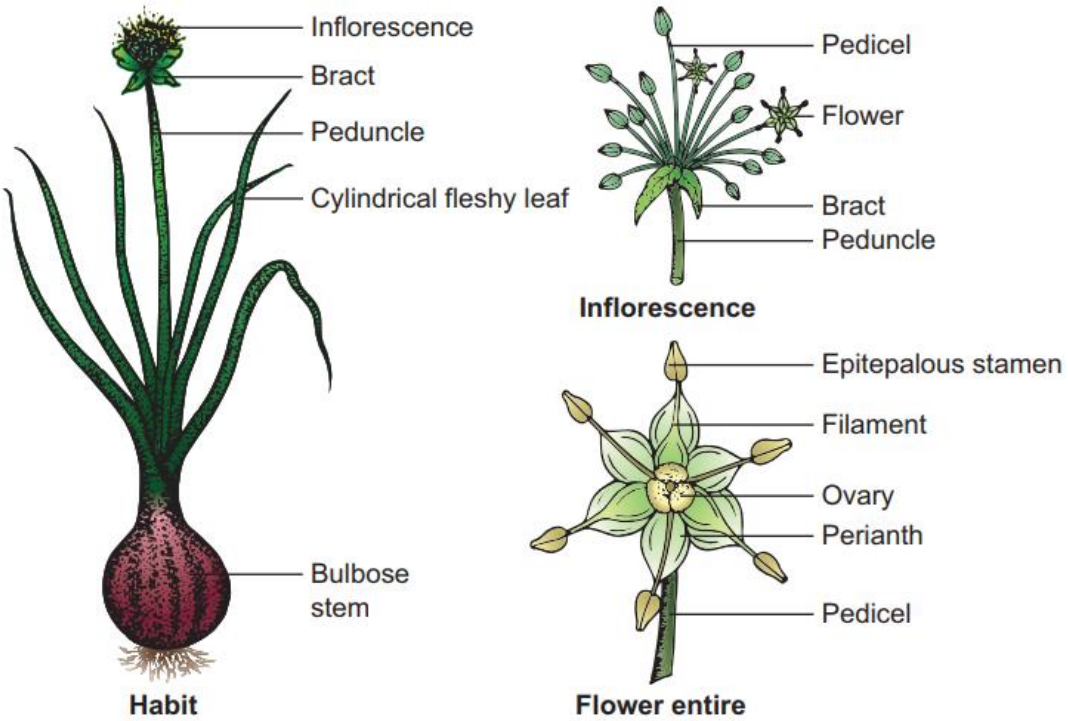


Fig: 1 Botanical description of Onion

Floral diagram of Onion:

Selfing and crossing in onion

CGMS based hybrid breeding in onion:

Experiment No. 11

Objective: To study about floral biology and selfing and crossing in pea

Botanical Name: ……. .

Chromosome No.: ……

Family: ……..

Pea plant is a common annual herb cultivated during the winter for the seeds. It is a weak plant and climbs with the help of tendrils. The roots are infected by nitrogen fixing bac-teria and they form characteristic nodules. Leaves are pinnately compound (imparipinnate), where the terminal leaflets are modified into tendrils. The leaf-base is swollen, forming pulvinus. A pair of foliaceous stipules is present.

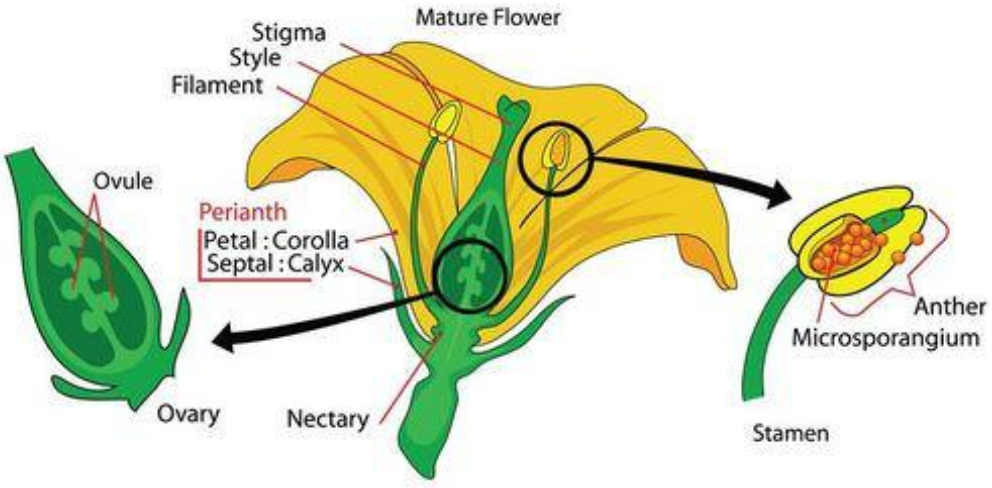
Floral biology: Flowers are lateral, solitary or in racemes. They are complete, irregular, zygomorphic, bisexual and slightly perigynous.
Calyx is composed of five united sepals.

Corolla is papilionaceous, made up of five free petals. The largest and the outermost one is the standard or vexillum, two lateral petals are wings or alae, and the innermost two, called keel or carina, unite to form a boat-shaped body. Aestivation is vexillary.

Androecium consists of ten stamens, nine united to form a bundle, one remaining free, diadelphous.

Gynoecium is monocarpellary, one-chambered with ovules in two series. Placentation is marginal. Ovary is elongated and superior.

Fruit is a legume dehiscing by both the sutures. Seeds are exalbuminous and germination is hypogeal. The plant is the sporophyte; the gametophytes, represented by pollen tube and embryo-sac, are extremely reduced and depen-dent on the sporophyte



Pea flower structure

Draw the floral diagram of pea:

Emasculation and Pollination techniques in pea:

Experiment No. 12

Objective: To study about induction of flowering in vegetable crops

Regulation of flowering and fruiting in tomato Flowers

- Normally the flower clusters are not pruned until 3 to 4 well-formed fruit appears on that cluster.
- However, abnormal flowers (the large fascinated flower) have to be removed as soon as recognized. This flower will produce a cat-faced fruit.

Terminal points

- Remove the terminal growing point above the top flower cluster (last cluster to pollinate) approximately forty five days before the intended date of terminating the tomato crop to stop plants from continuing to grow.
- Leave 2-3 leaves above the top cluster to shade and feed the top fruits.
- During hot summer months and leaving as many leaves on the plant can provide good shade for the growing fruit and cool the greenhouse.
- It is estimated that each plant will transpire close to 1-1.5 lit. of water a day and a house full of plants can lower the temperature of the greenhouse by at least 10 °F.

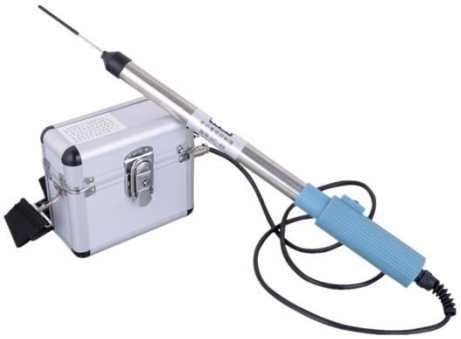
Pollination

- The female organs of the tomato flower are enclosed inside the male organs (five anthers attached together to form a cone around the female organ). Anthers open to the inside releasing pollen as soon as they mature.
- At maturity, the anthers will have a bright yellow colour and the flower will be receptive to pollination for about 48 hours.
- Pollen released without vibrating the flower will not be sufficient to produce high yield of good quality fruit. Field tomatoes are pollinated (vibrated) by natural wind.
- Because natural wind is absent in the greenhouse, tomato growers must pollinate their crop by several means including battery operated vibrators, air blowers, and bumblebees.
- Growers should also make every effort to transfer the maximum number of pollen to the stigma of the flower.
- The size and weight of the tomato fruit is positively correlated with the number of pollen transferred to the female part of the flower.

Battery operated vibrators

- Vibrators are small devices which can be purchased from any greenhouse supply store and operated by a weak electrical current from a battery.
- Vibrate the flowers by touching the stem of the flower cluster for few seconds. The strong vibration created by this tool will release more than enough pollen to fertilize the majority of the eggs in the ovary.
- Pollinate the flowers every-other-day on sunny days when humidity in the greenhouse is between 60 and 80%.
- Touch the cluster stem and do not touch the flower itself otherwise a hole will be created in the developing fruit. It takes approximately 30 minutes three times a week to pollinate 700 plants in one greenhouse of size 30X96 feet.
- This method of pollination is good for a small-size operation and the best method to guarantee pollinating every flower you want to pollinate and produce maximum-size fruit.

- Some of the drawback includes the fact that a grower has to be in the greenhouse at a certain time three times a week, it is a boring job, and the possibility of producing fruit with holes if you touch the flower.



- **Greenhouse Tomato Pollen Fertilization Machine Electric Bloom Pollinator Vibrating Tool**

Air blowers

- Greenhouse tomatoes can be pollinated by using a household leaf blower operated at normal speed with the air flow directed to the flower clusters.
- Use this device three times a week.
- It takes half the time to pollinate the same number of plants compared to the electric vibrator.
- However, the number of seed per fruit was less and fruit size and weight were smaller than fruit produced by using the vibrator for pollination.
- In general, anticipate five percent reduction in yield if you use this device.

Bumblebees

- Using bees to pollinate one or two greenhouses will save you time to do something else but it will not save money.
- Bumblebees are excellent pollinators for greenhouse tomatoes. Each bee will visit and vibrate the flower for few seconds to collect pollen for feeding. As a by product of this process, the stigma of the flower is showered with a large number of pollen leading to good pollination and fertilization of almost all the eggs in the ovary.
- Larger size and a heavier fruit is expected from bee pollination. Bees are active from sunrise to sunset, they do not take a long break or a day off.
- It is estimated that each bee can pollinate up to 350 flowers.
- Using a hive (even the smallest mini-hive) can lead to over pollination and injuring many flowers in a small greenhouse.
- Bumble bee box in polyhouse

Exercise: Use of Plant growth regulators in flowering induction in tomato

Regulation of flowering and fruiting in capsicum Flowering

Controlling fruit load

Regulation of flowering and fruiting in cucumber

Experiment No. 13

Objective: To study the techniques of inducing polyploidy

Introduction: Plants having more than two sets of chromosomes are referred to as polyploids. Auto-polyploids can be induced by chemical mutagen like colchicine, acenaphthene, caumarine etc. The colchicine is most widely used for chromosome doubling. Colchicines inhibit the formation of spindles fiber during metaphase. As a result, karyokinesis does not occur and the chromosome number of treated cell gets doubled.

Material required: Seeds, colchicines powder, beaker, petridish, cotton, brush and dropper.

Procedure:

1. Solution of colchicines (w/v) should be prepared in alcohol and required volume to be made in cold water.
2. Freshly prepared aqueous solution should be used because colchicine is unstable in aqueous solution.

Colchicines treatment may be imposed on seeds, seedlings or growing shoot apex.

Seed treatment: Soak the seeds in 0.2% solution for 7 days. Soak the seeds in shallow container to facilitate aeration (seed treatment may be done for 1 to 10 days with 0.001 to 1% solution).

Seedlings treatment: Young shoots in germinating seeds are dipped in 0.1% colchicines solution by inverting the seeds for 24 hour so that only young shoots are exposed to colchicines and roots are protected.

Shoot apex: Apply 0.5% colchicine solution with the help of brush or with a dropper in shoot apex of young seeding. Repeat the treatment twice daily for 5 days. Alternatively a small piece of cotton wool may be placed at the shoot tip, which daily soaked with 0.5% colchicines solution for 5 days.

Observation: 10 seedlings should be treated and to be observe

	Morphological characters (Treated plants)	Morphological characters (Untreated plants)
Seed treatment		
Seedling treatment		
Shoot apex treatment		

The observation for polyploidy to be recorded throughout the semester.

Experiment No. 14

Objective: To study the effect of mutagens to induce mutation

Chemical Mutation

Introduction: Among the chemical mutagens, the Ethyl Methane Sulphonate (EMS) is frequently used for in induction of mutation.

Material required: EMS, beaker, petridish, seed germinating trays, hand globes, seeds.

Procedure:

- Soak the seeds in distilled water for 1 hour at room temperature.
- Seeds are transferred to petridish containing 0.4% EMS solution in fully dipped position for 24hrs at room temperature.
- The seeds are rinsed in an automatic pipette washer for 30 minutes.
- Seeds should be sown immediately after the treatment.

Observation: 10 treated and 10 untreated seedlings to be transplanted for recording observation.

1. Percentage of germination

   Untreated_____

   Treated_____

2. Morphological characters (after 90 days)

		Seedling No.									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Height	Untreated										
	Treated										
Leaf area	Untreated										
	Treated										
Height at 1st flowering	Untreated										
	Treated										
Sex of flower	Untreated										
	Treated										
No. of flower	Untreated										
	Treated										

Physical Mutation

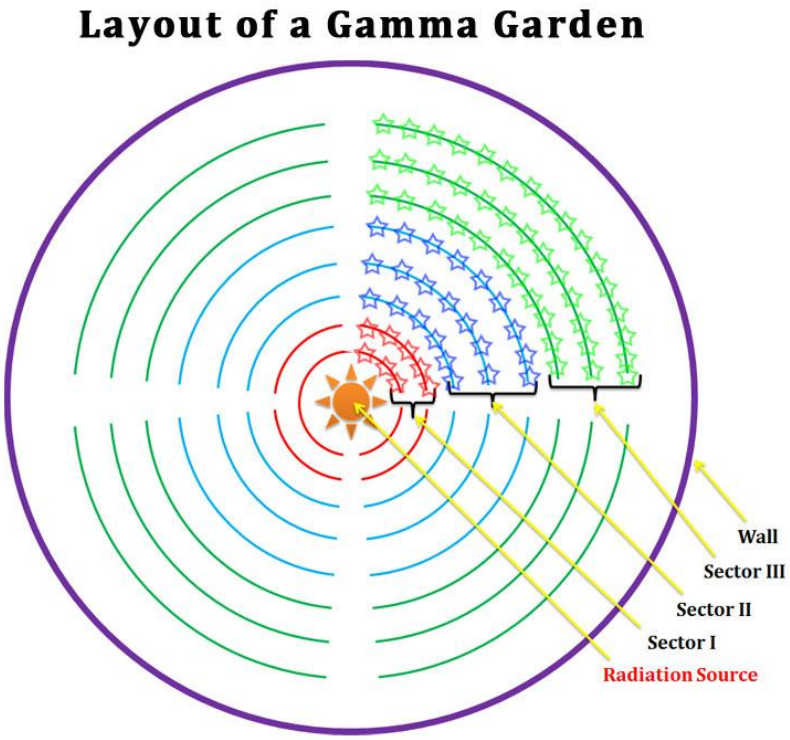
Gamma-Garden

Gamma gardens or atomic gardens are a type of induced mutation breeding where radioactive sources particularly gamma rays from cobalt -60 or Caesium-137 are used to induce desirable mutations in crop plants.

Salient features of Gamma Garden

- Rarely Caesium-137 is also used as the source of radiation.
- The strength of Co is 200 curies.
- The source of radiation is located at the centre.

- The area is divided into concentric circles with varying intensity of radiation.
- Plants to be irradiated are arranged as concentric circles around the radiation source.
- The intensity of radiation decrease as one move away from source of the radiation.
- The radially arranged plants in gamma garden can be grouped into three sectors.



1. The first gamma garden: in Long Island, New York, USA.
2. First gamma garden in India: Bose Research institute at Calcutta (1959).
3. Second gamma garden in India: Indian Agricultural Research Institute (1960).

Sector – I:
- They are plants nearest to the central radiation source.
- Plants in the sector-I usually die immediately due to the dose of radiation.
- They are not used in further experiments

Sector – II:
- This include plants located next to the sector-I
- These plants develop severe tumors, malformations and other abnormalities.
- These plants are also not used in further experiments.

Sector – III
- They include plants located next to sector II.
- They are the actual plants of interest in Gamma gardens.
- They may have random mutations not severe enough to damage the crop plant.
- The variations obtained in the sector III are used in further breeding experiments.
- They can be used as a source of variation in hybridization or can be directly released as a variety.

Advantages of Gamma Garden
- Gamma gardens can produce large amount of variations within a short time.
- Desirable mutants can be released directly as a new variety.
- Gamma gardens are good examples of the peaceful use of atomic energy for human welfare.

Disadvantages of Gamma Garden:
- High initial investment required.

- Other cheapest mutation methods are now available.
- Chances of undesirable mutations are very high.
- Mutations are random; we cannot predict the effects of mutations.

Exercise:

Write the name of mutant varieties:

Tomato

Chilli

Okra

Palak

French Bean

Potato

Brinjal

Amaranth

Experiment No. 15

Objective: Screening techniques for abiotic stress resistance in vegetable crops

Screening for drought resistance

Drought refers to the condition of moisture deficiency. Soil drought is more common in the arid and semi-arid tropics and in the areas of steep slope. Thus desert areas are more prone to drought conditions. Drought resistance refers to the ability of crop plants to grow, develop and reproduce normally under moisture deficit conditions.

Drought hardening

Improvement in the drought tolerance ability of a genotype through various treatments is referred to as drought hardening. Two types of treatments are in common use for drought hardening. These are pre-sowing treatment and post-sowing treatment.

Pre-sowing treatment

Seeds are treated before sowing to induce drought hardiness. Seeds are soaked in water for 24 hours and then sun dried. The treated seeds are sown in the field. Plants developed from such seeds exhibit improved drought resistance or tolerance. The hardened plants exhibit higher yield under drought, higher water contents, increased viscosity of protoplasm, more bound water, higher metabolic rate and stronger roots. The same seed treatment, in sunflower, resulted in more stable metabolism of nitrogen and phosphorus, besides higher yield under drought conditions than untreated plants. Various modifications of seed treatment method have been suggested by various workers.

Post-sowing treatment

The post-sowing treatment is given to the young seedlings. Mild drought stress in early stages of growth increases resistance to water stress under subsequent and more severe drought. Several cycles of stress helped in identification of the most drought resistant and the most susceptible genotypes by measuring their survival.

Measurement of drought resistance

Various procedures are used for measuring drought resistance in crop plants. The most commonly used procedures include (1) water retention, (2) photosynthesis, (3) yield performance, and (4) root length of seedlings. 1these techniques can be used age scale screening of segregating populations in breeding programme.

1. Leaf water retention

In this method leaves are excised from the plant and are allowed to dry. The slower drying genotypes are considered as drought tolerant In other words, the high water retainer. Genotype are considered as drought tolerant Some instigators use tissue water potential as an index of water stress under drought conditions. The tissue water potential is measured with the help of thermocouple psychrometer. The portable field psychrometer is widely used for measuring drought resistance in segregating populations.

2. Rate of photosynthesis

The ratio of photosynthesis during and after moisture stress is an important index of drought resistance. Now portable non destructive photosynthesis analyzed are available which can be used in the fields for large scale screening of germplasm as well as segregating populations in standing crops. The genotypes which have high photosynthetic rate under moisture stress are considered as drought resistant, because such genotypes give higher yield than those having low photosynthetic rate. A simple portable photosynthesis analyser made it possible to measure

photosynthesis of many plants within a short time. Now photosynthesis is used as a criterion to select for drought.

3. Yield performance

Superior yield performance under moisture stress conditions is an important and reliable index of drought tolerance. The yield tests should be conducted in drought prone areas at several locations or for several years. These will help in identification of genotypes with drought resistance and also in the elimination of drought susceptible lines. The yield test should be conducted under both fields as well as glasshouse conditions. Moreover, large number of populations should be grown. This will enhance chances of obtaining superior drought resistant genotypes.

4. Root length of seedlings

The root length during seedling stage is also used as a measure of drought resistance. ln a more recent study, it was observed that root mass after 30 days was reliable index of root mass at maturity. Thus those genotypes which have longest root during seedling stage also exhibit extensive root system at maturity. This is simple and quick method of measuring drought resistance. Several thousand plants are screened for seedling root length in the greenhouse in each crop season. Moreover, after screening superior plants can be replanted and grown to maturity. Some workers use hydroponics tank to measure the root growth of seedlings.

Exercise:

Screening techniques for salinity in tomato:

Screening techniques for heat tolerance in tomato:

Experiment No. 16

Objective: Screening techniques for disease resistance in vegetable crops

Introduction: Crop plants are infected by various fungal, viral and bacterial diseases. Screening for disease resistance is carried out under field and glasshouse conditions. Glasshouse tests are conducted under controlled conditions and therefore, glasshouse screening is considered more reliable than field tests. However field and glasshouse screenings are equally important.
The procedure of field inoculation differs from various types of diseases. Diseases are classified into four main groups.

1. **Soil borne diseases:** For soil borne diseases like root rots, collar rots, wilts etc. the screening is done in disease sick plots. Disease sick plots are developed in following three ways:
   - **a.** By mixing soil from other sick plots.
   - **b.** By adding remains of diseased plants in the soil or plot which is to be developed into wilt sick plot.
   - **c.** By adding inoculums developed in the laboratory.

   Breeding material for screening purpose can be planted in the disease sick plots. These plants which remain healthy in disease sick plots are selected and further tested under glasshouse conditions to confirm the resistance. The soil from the disease sick plots can be used in pots for conducting tests in the glasshouse.
2. **Air borne diseases:** Air borne diseases include rusts, smuts, mildews, blights, leaf spots etc. For such diseases, the screening is done either by dusting the spores or by spraying the spore suspension on the healthy plants. Planting of highly susceptible varieties after few rows of test material or all around the test material is also used to develop the inoculums. The healthy plants are identified and selected.
3. **Seed borne diseases:** Seed borne diseases include smut and bunts. For such diseases either dry spore are dusted on the seeds or the seeds are soaked in the spore suspension and then used for planting. After such treatment, those plants which remain healthy are identified are selected.
4. **Insect transmitted diseases:** Generally diseases caused by viruses are transmitted through insects. For screening against such diseases, insects from susceptible varies are collected and released on healthy plants or juice of diseased plants is rubbed on the healthy plants after causing mechanical injury in healthy plants. After such treatment, these plants which remain healthy or do not develop disease are identified and selected.

0	0	0	0	x	x	0	0	0	0	x	x	0	0	0	0
0	0	0	0	x	x	0	0	0	0	x	x	0	0	0	0
0	0	0	0	x	x	0	0	0	0	x	x	0	0	0	0
0	0	0	0	x	x	0	0	0	0	x	x	0	0	0	0
0	0	0	0	x	x	0	0	0	0	x	x	0	0	0	0
0	0	0	0	x	x	0	0	0	0	x	x	0	0	0	0
0	0	0	0	x	x	0	0	0	0	x	x	0	0	0	0
0	0	0	0	x	x	0	0	0	0	x	x	0	0	0	0

Fig.: Susceptible line adjacent

0 = Test material

X = Susceptible line

x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
x	x	0	0	0	0	0	0	0	0	0	0	0	0	x	x
x	x	0	0	0	0	0	0	0	0	0	0	0	0	x	x
x	x	0	0	0	0	0	0	0	0	0	0	0	0	x	x
x	x	0	0	0	0	0	0	0	0	0	0	0	0	x	x
x	x	0	0	0	0	0	0	0	0	0	0	0	0	x	x
x	x	0	0	0	0	0	0	0	0	0	0	0	0	x	x
x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

Fig.: Susceptible line all around

Exercise:

Screening techniques for black rot resistance in cauliflower:

Black rot symptoms:

Disease score:

Screening techniques:

Experiment No. 17

Objective: Molecular marker techniques to identify useful traits in the vegetable crops

The protocol for extraction of DNA and the primary objective is development of relatively quick, inexpensive and consistent protocol to extract high quality DNA with better yield from expanded leaf material containing large quantities of polyphenols, tannins and polysaccharides. The general principle of DNA extraction is disruption of the cell wall, cell membrane and nuclear membrane to release the highly intact DNA into solution followed by precipitation of DNA while ensuring removal of the contaminating biomolecules such as the proteins, polysaccharides, lipids, phenols and other secondary metabolites by enzymatic or chemical methods.

Solutions

1. Homogenization buffer:
200 mM Tris-HCl
50 mM EDTA
2.2 M NaCl
2% CTAB
0.06% sodium sulphite
pH8.0

2. Phenol: Chloroform: Isoamyl alcohol (25:24:1)

3. 6 M NaCl

4. 10% Polyvinylpyrrolidone (PVP)

5. 5% N-lauroyl-sarcosine

6. 20% CTAB

7. TE (10 mM Tris-HCl, 1mMEDTA, pH 8.0).

CTAB based DNA isolation protocol:

Note

- To quantify DNA concentration, uncut λ genomic DNA of known concentration (25, 50, 100, 150, 200) should be used during electrophoresis.
- The gel image should be checked for the presence of any kind of RNA contamination or DNA degradation in the samples

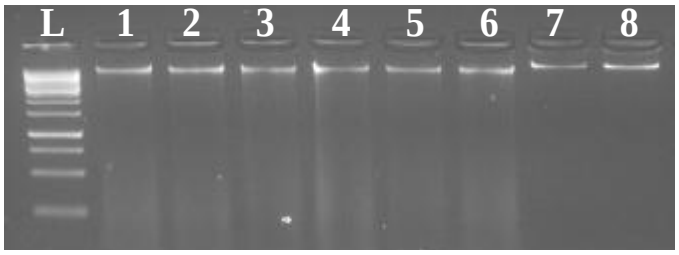


Fig.: Gel picture showing genomic DNA of diluted Potato checked on 0.8% Agarose L: 1Kb DNA ladder; 1-6 (Tomato genotypes): T1, T2, T3, T4, T5, T6, 7: 50 ng λ DNA; 8: 100 ng λ DNA

PCR: principle and procedure

The polymerase chain reaction (PCR) is basically defined as a scientific technique in molecular biology which is used to amplify a single or a few copies of a DNA fragment to several orders of magnitude thereby generating millions of copies of a particular DNA sequence. Kary B. Mullis invented PCR technique in 1983 which is hailed as one of the greatest innovations that revolutionized the modern filed of molecular biology. PCR takes analysis of tiny amounts of genetic material; even damaged one to a new level of precision and reliability.

PCR Requirements:

1. RAPD primers
2. High quality DNA of working standard: 20 - 30 ng/µl
3. Taq DNA polymerase (1 unit/reaction)
4. dNTPs (200 µM each)
5. Buffer
6. Nuclease free autoclaved distilled water
7. $MgCl_2$

PCR reaction mix

- Buffer (Containing 1.5mM $MgCl_2$) 2.5 µl
- dNTPs (2.5mM each) 2.0 µl
- Primer (200-500 pg) 1.0 µl
- DNA template (~50 ng) 1.0 µl

- Taq DNA polymerase (5U/µl) 0.2 µl
- $MgCl_2$ 0.5 µl
- Nuclease free water 17.8 µl

Total Volume/reaction 25.0 µl

PCR conditions:

- 95^0C :5 min (Initial Denaturation)
- 95^0C : 40 s ⎫ (Denaturation)
- 50^0C : 40 s ⎬ 35 cycles (Annealing)
- 72^0C* : 2 min ⎭ (Extension)
- 72^0C : 12 min (Final Extension)
- 4^0C : ∞ (Hold)

Where, * is Extension carried out at 72^0C for 2 min +3 s extension/cycle

Types of molecular markers:

Dominant and co-dominant markers:

Experiment No. 18

Objective: Screening techniques for insect-pests in vegetable crops

Principle: Resistance of plants to insects enables a plant to avoid or inhibit host selection, inhibit oviposition and feeding, and reduce insect survival and development, tolerate, or recover from injury from insect populations that would cause greater damage to other plants of the same species under similar environmental conditions.

The following techniques can be used to screen for plant resistance to insects.

Manipulations of Insect Abundance/Density

It is possible to manipulate insect abundance by field infestation, caging, artificial rearing, and by evaluating insecticide-protected and unprotected plots.

Field infestation:

Caging:

Supplementing natural abundance with artificially reared insects: Artificially reared insects can be made available throughout the year for screening tests. Artificial diets have been developed for several insect species. In sorghum, e.g., spotted stem borer can be reared on an artificial diet. But, if it is not possible to rear insects on an artificial diet, insect colonies can be maintained on natural hosts (shoot fly, head bugs, and midges) under greenhouse conditions.

Measurement of Resistance

Direct-feeding injury:

Simulated feeding injury:

Correlation of plant factors with insect resistance:

Indirect Measurements of Resistance

Sampling insect populations:

Measurements of insect feeding and development:

Measurements of insect behavior:

Screening against whitefly in tomato: